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Introduction

The development of resistance to radiation and chemotherapeutic agents that cause DNA damage is a major problem for the treatment of breast cancer (1), which argues for the development of new therapeutic agents that can either augment the effects of radiation and chemotherapy or that can be applied as an adjunct or alternative treatments. One promising new treatment modality is the application of vector-mediated gene therapy. A noted problem with many vectors, including both viral and non-viral vectors, used for gene therapy is the lack of efficient and targeted delivery to the primary tumor and disseminated metastases. To address this issue, we propose the use of CD34⁺ and/or Flk-1⁺ endothelial progenitor cells (EPCs), which have the propensity of homing to sites of neoangiogenesis. Key to the success of this approach is a vector system for the efficient genetic modification of the EPCs. In this regard, we have shown previously that CD34⁺ EPCs are efficiently transduced using herpesvirus vectors with relatively low multiplicity of infections and toxicity. Given this capacity to efficiently infect EPCs with herpesvirus vectors, it is our hypothesis that EPCs can be genetically modified and, after intravascular injection, these normally circulating cells will localize into sites of tumor angiogenesis. Further, the natural targeting capacity of EPCs will allow their use as cellular vehicles for gene therapy of both local and disseminated breast cancer.

Body

On 9/14/00 the Principle Investigator responsibilities for this project were re-assigned to Dr. Jerry L. Blackwell. I maintained an interest during the early developmental phases of the project and enthusiastically endorsed taking over the project when Dr. Jesus Gomez-Navarro left the University of Alabama and could no longer service the grant. Since taking over the Principle Investigator responsibilities I have assigned a highly experienced research assistant, Dr. Hui LI, to work on this project. Dr. Hui currently has ~50% effort dedicated to this project.

Although Dr. Hui and I have extensive experience in adenovirus-based gene therapy using various *in vitro* and *in vivo* models, we have had to devote a

considerable amount of time in developing the herpesvirus purification and experimental protocols. We have now developed the protocols for large scale herpesvirus purification and have recently produced enough herpesvirus for *in vivo* experimentation.

In addition of developing a protocol for large scale herpesvirus purification, we have also focused on the isolation of various blood-derived cell types. Our initial attempt to isolate CD34⁺ EPCs from human peripheral blood mononuclear cells following leukapheresis yielded a highly enriched CD34⁺ EPCs preparation, but it was of insufficient quantity for *in vivo* applications. Since it is ill-advised to perform frequent leukapheresis on the same donor (less than 12 times per lifetime), we are currently evaluating alternative technologies for the isolation of CD34⁺ EPCs from fresh donor blood without leukapheresis. In this regard, we have recently tested the StemSep system (StemCell Technologies, Inc.) and achieved high purification (~77-fold enrichment, 30% recovery) of CD34⁺ starting with $\sim 1 \times 10^8$ ficoll cells.

As proof-of-principle in our capacity to genetically modify blood-derived cells, we have recently generated dendritic cells from peripheral mononuclear cells and infected them *ex vivo* with another viral vector, in this case, adenovirus. Briefly, dendritic cells were generated as described elsewhere (2) after which they were infected with adenovirus or adenovirus plus a CD40-targeting fusion protein (manuscript in preparation). The CD40-targeting protein molecule has two binding specificities. One half of the molecule contains the ectodomain of the native Ad receptor, CAR, which facilitates binding to the Ad fiber protein. The other half of the molecule contains a single-chain antibody that recognizes the CD40 receptor, which provides the targeting capacity to the CD40 receptor on the surface of dendritic cells. The CAR ectodomain and CD40-sFv are genetically fused together with a linker sequence. In the absence of the targeting protein, Ad inefficiently infects dendritic cells; however, when coated with the CD40-targeting fusion protein, Ad efficiently infects dendritic cells from both human and rhesus macaque monkeys (Figure 1). Gene transfer efficiency was enhanced by 176-fold in the human dendritic cells (Figure 1, left panel). In addition, pre-incubation of rhesus macaque dendritic cells with an anti-CD40 antibody blocked the augmentation of gene transfer using the CD40-

targeting fusion protein (Figure 1, right panel), thereby showing the CD40 receptor specificity of the targeted vector.

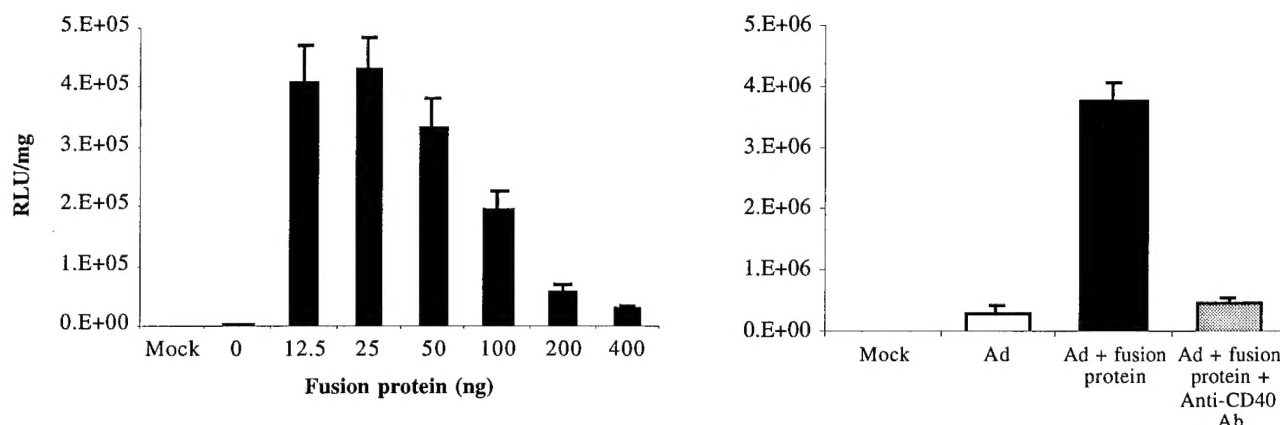
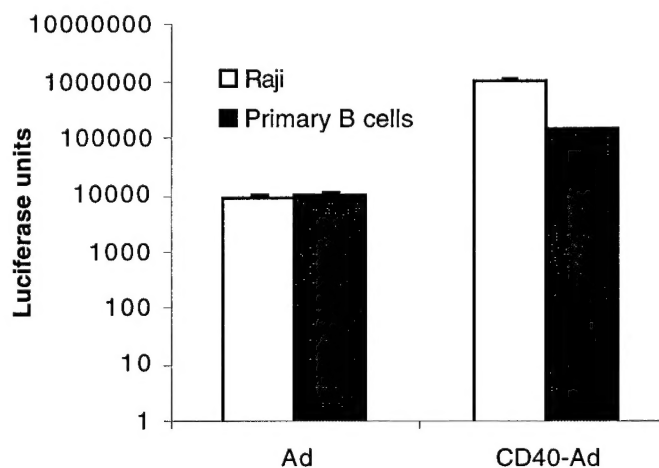


Figure 1. CD40-targeting fusion protein augments adenovirus gene transfer to dendritic cells. Adenovirus was incubated in solution with different amounts of the CD40-targeting fusion protein for 30 minutes on ice. Left panel. The CD40-targeting fusion protein was titrated with a constant amount of adenovirus. Human dendritic cells infected with the CD40-targeted adenovirus and 24 h later luciferase activity was measured. Right panel. Rhesus macaque dendritic cells were transduced with the CD40-targeted adenovirus. One group of the dendritic cells was preincubated with anti-CD40 antibody prior to infection. 24 h later the cells were lysed and luciferase activity was measured.

We also evaluated recently the capacity of the CD40-targeting fusion protein to target CD40⁺ another candidate cell vehicle, primary B cells and B cell lymphoma (Raji). In both case the CD40-targeting fusion protein significantly augmented transduction (Figure 2).

Figure 2. CD40-targeting augments gene transfer to B cells. Adenovirus was incubated with an optimal concentration of the CD40-targeting fusion protein for 30 minutes on ice. Left panel. Primary human B cells and a B cell lymphoma cell line (Raji) were then infected with the CD40-targeted adenovirus and 24 h later luciferase activity was measured



Collectively these data (shown in Figures 1 and 2) demonstrate the ability to specifically target candidate cellular vehicles using a viral vector. In addition, targeting

receptors present on the cell vehicles dramatically augments the transduction efficiency of the vectors. Similar types of receptor targeting may be possible in the case of CD34⁺ EPCs using herpesvirus vectors as well as adenovirus vectors. This finding has several important ramifications in the current project: (1) targeting to receptors on the cell vehicles allows for much more efficient transduction, (2) higher transduction efficiency allows for the utilization of lower vector concentrations to achieve comparable levels of transduction with untargeted vectors, (3) lower vector concentrations generally correlated to less toxicity, and (4) receptor-specific targeting allows for the possibility of *in vivo* targeting of cell vehicles.

Key Research Accomplishments

- Protocol development for large scale herpesvirus purification
- Protocol development of highly enriched CD34⁺ EPCs preparation from human peripheral blood mononuclear cells
- Demonstration that receptor targeting dramatically improves cell vehicle transduction

Reportable Outcomes

Since taking over this project our primary focus has been on protocol development. We have extensive experience with mouse model systems and do not anticipate additional “downtime” as we enter the experimental phase of the project. Experiments are currently underway to investigate the *in vivo* biodistribution patterns of CD34⁺ EPCs in a tumor-bearing mouse model. I anticipate this to be the initial reportable outcome from this project.

Conclusions

As pointed out in the previous section, we have had some down time due to problems associated with large scale vector purification and isolation of sufficient quantities of CD34⁺ EPCs. I am confident that both problems are now remedied and we will rapidly advance the main goals of the project.

The capacity to target specific receptors on cellular vehicles, such the CD40 targeting with dendritic cells and B cells (above), has direct relevance to the current project. Receptor targeting allows for the use of less vector, results in lower toxicity caused by the vector, and renders the vector more efficacious. Parallel studies will commence to evaluate the ability to target specific receptors on CD34⁺ EPCs.

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